

COMPOUNDS WHICH CAN BE USED TO DIAGNOSE AND MONITOR DISEASES  
ASSOCIATED WITH THE FORMATION OF AMYLOID PROTEIN FIBRILS

Field of the Invention

5           The present invention refers to compounds which can be  
used to diagnose and monitor diseases associated with the  
formation of amyloid protein fibrils, to their use in the  
diagnosis of said diseases, to pharmaceutical compositions  
containing said compounds, and finally, to methods of  
10       preparing said compounds.

Background of the Invention

15           It is known that diseases such as Alzheimer,  
Parkinson's, Huntington and Creutzfeld-Jacob, cystic fibrosis,  
late onset diabetes and motor neuron disease, amongst others,  
occur with the formation of amyloid protein fibrils from  
precursor proteins. These fibrils may present themselves in  
systemic form or as localised insoluble deposits. It is  
currently accepted that fibril forming amyloid proteins are  
characterised by being positive for Congo Red, being fibrillar  
20       by electron microscopy, and having a crossed beta structure by  
X-ray diffraction. More than 20 amyloid protein precursor  
proteins are known, some examples being light and heavy chain  
immunoglobulins, transthyretin, apolipoprotein A1, gelsolin,  
lysozyme, the A $\beta$  precursor protein, prion proteins, the atrial  
25       natriuretic factor, prolactin and insulin.

30           Abnormal accumulations of two types of amyloid protein  
deposits in brain regions involved in learning tasks and  
memory, such as the hippocampus, are observed specifically in  
patients with Alzheimer's disease after *post-mortem*  
examination. Of these two types, the extracellular amyloid  
plaques are the most characteristic of Alzheimer's disease,  
while the intracellular neurofibrillary tangles may also be  
found in other types of neurodegenerative diseases.

35           The amyloid plaque is made up of dystrophic neurites and  
other altered astrocytes, as well as microglia which envelopes

an insoluble fibrillar core. These fibrils are made up of a series of proteins which are generically called  $\beta$ -amyloid proteins, two of which, A $\beta$ 40 and A $\beta$ 42 are predominant. It has also been demonstrated that certain transition metals are intrinsic of the composition of  $\beta$ -amyloid aggregates, given that  $\beta$ -amyloid proteins have the ability to bond metals through specific locations for Cu and Zn. This bond is the one that mediates the precipitation of these proteins and, therefore, high concentrations of metals such as Cu and Zn are found in the neocortex and even higher concentrations in the  $\beta$ -amyloid plaques of Alzheimer patients.  $\beta$ -amyloid proteins are generated from corresponding precursor proteins which are expressed in a ubiquitous manner on cell surfaces. It is accepted today, although there is no irrefutable proof thereof, that the formation and accumulation of  $\beta$ -amyloid proteins, be it in prefibrillar or diffusible form or as plaque itself, is a starting and necessary factor in Alzheimer pathogenesis, which, in turn, precedes neurodegeneration. There is also a consensus in that therapeutic strategy design must be geared towards these processes.

In daily medical practice, neuropsychological examination and clinical observation of symptoms such as cognitive decline and systematic elimination of other possible causes of said symptoms is the standard and universally recognised method for determining whether a patient has probabilities of being affected by Alzheimer's disease or of already being in a first symptomatic stage (minimum symptoms of invalidity). The only way today of confirming these diagnoses with complete certainty is *post-mortem* brain examination. In these examinations, synapse counting is the most precise marker. However, the amount of amyloid plaque deposited is approximately correlated with the severity of the symptoms at the time of death of the patient. More specifically, it has been observed that the increase in the

total amount of  $\beta$ -amyloid protein 1-42 in the hippocampus and in some cortical regions correlates perfectly with the early onset of clinical symptoms and their subsequent progression.

5 In this context, the possibility of obtaining functional images of the amount and distribution of amyloid plaques could serve as an important complement to the diagnosis of presymptomatic stages of the disease. Furthermore, the development of new *in vivo* brain amyloid deposit neuroimaging techniques could be much more transcendental for the  
10 evaluation of therapeutic alternatives. More specifically, given the need to test therapeutic agents that may inhibit the production of or redissolve  $\beta$ -amyloid proteins and thus reduce or prevent development of the disease it is crucial, for example, to be able to make informed decisions on matters such  
15 as: what type of patients are admitted in clinical tests, when the treatment is applied and how the progress of the treatment is evaluated.

In a chronic and non-infectious disease such as Alzheimer's disease, it is logical to assume that the first  
20 clinical trials of new substances potentially able to modify the course of the disease must be therapeutic rather than preventive. The results of these first trials have already been presented at the 8<sup>th</sup> International Conference on Alzheimer's Disease (Stockholm, 20-25 July 2002) and a summary  
25 thereof has been published in Science magazine (*New Alzheimer's treatments that may ease the mind*, Science, 297, 1260-1262). It is urgent, therefore, for these and other efforts not to be in vain, to develop sensitive and precise methods that allow to assess the progress or regression of the  
30 disease during pharmacological treatment in a manner as objective and as quantitative as possible.

Amongst the imaging diagnostic methods available for this type of monitoring the Non-Invasive Radiotracer Imaging (NIRI) techniques are very suitable, as they allow visualising  
35 and quantifying specific events at molecular level such as

those involved here. Of these techniques, there are two methods that provide high resolution images, one that uses positron emitting isotopes such as  $^{18}\text{F}$  and  $^{11}\text{C}$  (Positron Emission Tomography: PET) and one that uses simple photon emitting radionuclides such as  $^{99\text{m}}\text{Tc}$  and  $^{123}\text{I}$  that allow obtaining planar images or images by Single Photon Emission Computerised tomography (SPECT).

Two research strategies, among others, are currently directed towards achieving a method that allows assessing the progress of Alzheimer's disease during a pharmacological treatment, but none of these have yet corroborated their strategy in systems *in vivo*. One of these consists in the intravenous administration of radioiodinated ( $^{125}\text{I}$ )  $\beta$ -amyloid protein which, when passing through the blood-brain barrier (BBB) may incorporate itself onto the neuritic plaques of the brain and thus allow its locating. This approach presents several problems such as, for example, that proteins are susceptible to degradation, that they have problems going through the blood-brain barrier, that they penetrate tissue with difficulty and that they are difficult to produce in high quantities. In this field, the most important efforts have been geared towards improving the permeability of  $\beta$ -amyloid proteins with regard to the BBB. One of the first strategies has consisted in joining the  $\beta$ -amyloid protein to protein vectors for which specific transporters exist in the BBB (Saito Y., et al.; *Vector-mediated delivery of  $^{125}\text{I}$ -labeled  $\beta$ -amyloid peptide A $\beta$ 1-40 through the blood-brain barrier and binding to Alzheimer's disease amyloid of the A $\beta$ 1-40/vector complex; Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 10227-10231*). In more recent approaches these vectors are the natural polyamines spermine and putrescine. It has been observed that the radioiodinated  $\beta$ -amyloid peptide (1-40) with putrescine conjugates bond better than the non-conjugated form and almost exclusively to the dense amyloid deposits of neuritic plaques

in rats. This bonding does not take place on the innumerable diffuse deposits of  $\beta$ -amyloid protein that are not surrounded by dystrophic neurites and which, as would be desirable, could be indicators of precocious lesions (Wengenack T.M. et al.; "Targeting amyloid plaques in vivo"; Nat. Biotechnol. 18 (2000) 868-872).

The other main line of research proposes the use of colorants with *in vitro* affinity for amyloid plaques as radiopharmaceutical tracers. One of the first jobs with Chrysamine G, an amyloid colorant which penetrates the BBB, has demonstrated the viability of this approach by staining amyloid plaques with technetium in different brain regions. Other researchers have tried to redesign colorants such as Congo Red in order to accommodate in its molecule radioisotopes such as  $^{99m}\text{Tc}$ , which have been demonstrated to be useful to visualise amyloid lesions *in vitro* (Zhen W.; "Synthesis and amyloid binding properties of rhenium complexes: preliminary progress toward a reagent for SPECT imaging of Alzheimer's disease brain" J. Med. Chem. 42 (1999) 2805-2815). One of the last attempts has been performed based on a new fluorescent colorant called BSB [(trans, trans), -1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene]. BSB is capable of bonding to amyloid plaques in transgenic mice but it also has affinity for other  $\beta$ -sheet rich protein deposits such as intraneuronal neurofibrils and other bodies such as Lewy bodies which are common to other neurodegenerative diseases such as Parkinson's (Skovronsky D.M. et al.; *In vivo* detection of amyloid plaques in a mouse model of Alzheimer's disease; Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 7609-7614).

On the other hand, US patent US 4 360 509 discloses the use of radioactive indium and 8-hydroxyquinoline chelates to locate inflammatory reactions in hot blooded animals by means of non-invasive imaging techniques. International patent application WO 00 76966 discloses rhodamine derivatives

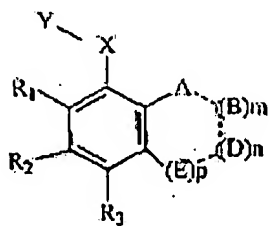
labelled with radioisotopes which are used for the diagnosis of diseases related to the amyloid protein deposition.

There is, therefore, a need for the identification and preparation of new alternative compounds that are useful for diagnosis and monitoring of diseases related to protein deposition in the central nervous system and which, furthermore, do not present the disadvantages of the compounds already used in the state of the art.

#### Summary of the Invention

After exhaustive and laborious research, the applicant has prepared some compounds which, due to their structure are capable of interacting with the amyloid protein fibrils, including those which appear as amyloid plaques and affect the central nervous system, and which are surprisingly also capable of going through the blood-brain barrier. Said compounds are, therefore, useful for the diagnosis and monitoring of diseases associated with the formation of amyloid protein fibrils and, furthermore, do not present the drawbacks of the compounds and methods used up to now in the state of the art, such as the degradation of said compounds, their low permeability with respect to the blood-brain barrier, their low specificity, etc. With these compounds it is therefore possible to effect a diagnosis or a monitoring of the aforementioned diseases in animals, transgenic animals and, particularly, in humans.

One aspect of the present invention consists in the use of the compounds of General Formula I



Formula I

wherein:

X represents O, S;

Y represents H or, along with X, where  $X = O$ , a carbohydrate radical;

5 A represents N or  $NR_4$ ;

B represents  $CR_5$ ,  $NR_5$  or N;

D represents  $CR_6$ ,  $NR_6$  or N;

E represents  $CR_7$ ,  $NR_7$  or N;

10 with the condition that the ring containing group A has a maximum of two nitrogen atoms in its structure;

m, n and p represent: 0 or 1, where  $m + n + p = 2$  or 3;

15 the dashed lines - - - represent a single or double bond;

$R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$  and  $R_7$ , each independently represent a radioactive isotope, H, a halogen or a radical optionally having a radioactive isotope, said radical being chosen from:  $C_1$ - $C_6$  alkyl, OH,  $C_1$ - $C_6$  polyhydroxyalkyl,  $C_1$ - $C_6$  alkoxyl,  $C_1$ - $C_6$  alkoxyalkyl,  $(CH_2)_q$ -OR', wherein q is 1, 2 or 3,  $CF_3$ ,  $CH_2$ - $CH_2$ F,  $O$ - $CH_2$ - $CH_2$ F,  $CH_2$ - $CH_2$ - $CH_2$ F, CN,  $NO_2$ ,  $O(CO)R'$ ,  $OR'$ ,  $SR'$ ,  $COOR'$ ,  $-SO_3H$ ,  $(CH_2)_r$ - $CO_2R''$ ,  $(CH_2)_r$ -CO-R', wherein r is 1, 2 or 3 and Rph, wherein Rph represents a non substituted or substituted phenol group, the possible substituents of the phenol group being any of the meanings of  $R_1$ - $R_7$ , except for a phenol group;

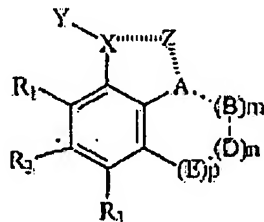
25  $R'$  is H or a  $C_{1-3}$  alkyl group;

30  $R''$  is H, a  $C_1$ - $C_6$  alkyl group or a  $C_1$ - $C_6$  alkyloxy group;

35 with the condition that only one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X and Y is or has a radioactive isotope; in the preparation of a composition for diagnosis and/or monitoring of diseases associated with the formation of amyloid protein

fibrils, particularly those that appear as amyloid plaques and affect the central nervous system.

A second aspect of the present invention consists in the use of the compounds of General Formula II.



Formula II

wherein:

X represents O, S;

Y represents H or, along with X, where X = O, a carbohydrate radical;

Z represents a metal or rare earth cation that may or may not be radioactive;

the ||||| line represents a coordinate bond;

A represents N or NR<sub>4</sub>;

B represents CR<sub>5</sub>, NR<sub>5</sub> or N;

D represents CR<sub>6</sub>, NR<sub>6</sub> or N;

E represents CR<sub>7</sub>, NR<sub>7</sub> or N;

with the condition that the ring containing substituent A has a maximum of two nitrogen atoms in its structure;

m, n and p represent: 0 or 1, where m + n + p = 2 or 3;

the dashed lines - - - represent a single or double bond;

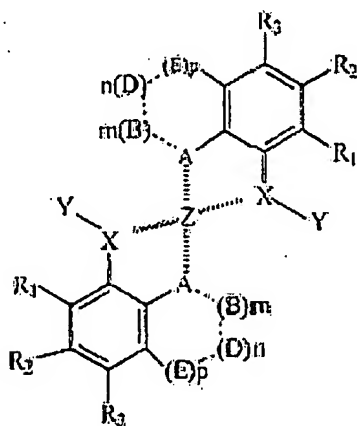
R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub> and R<sub>7</sub> each independently represent a radioactive isotope, H, a halogen or a radical optionally having a radioactive isotope, said radical being chosen from: C<sub>1</sub>-C<sub>6</sub> alkyl, OH, C<sub>1</sub>-



$C_6$  polyhydroxyalkyl,  $C_1-C_6$  alkoxyl,  $C_1-C_6$  alkoxyalkyl,  $(CH_2)_q-OR'$ , wherein  $q$  is 1, 2 or 3,  $CF_3$ ,  $CH_2-CH_2F$ ,  $O-CH_2-CH_2F$ ,  $CH_2-CH_2-CH_2F$ ,  $CN$ ,  $NO_2$ ,  $O(CO)R'$ ,  $OR'$ ,  $SR'$ ,  $COOR'$ ,  $-SO_3H$ ,  $(CH_2)_r-CO_2R''$ ,  $(CH_2)_r-CO-R'$ , wherein  $r$  is 1, 2 or 3 and Rph, wherein Rph represents a non substituted or substituted phenol group, the possible substituents of the phenol group being any of the meanings of  $R_1-R_7$  except for a phenol group;  $R'$  is H or a  $C_{1-3}$  alkyl group;  $R''$  is H, a  $C_1-C_6$  alkyl group or a  $C_1-C_6$  alkyloxy group;

with the condition that only one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $X$ ,  $Y$  or  $Z$  is or has a radioactive isotope; in the preparation of a composition for diagnosis and/or monitoring of diseases associated with the formation of amyloid protein fibrils, particularly those that appear as amyloid plaques and affect the central nervous system.

A third aspect of the invention consists in the use of the compounds of General Formula III.



Formula III

wherein:

$X$  represents O, S;

Y represents H or, along with X, where  $X = O$ , a carbohydrate radical;

Z represents a metal or rare earth cation that may or may not be radioactive;

5 the ||||| line represents a coordinate bond;

A represents N or  $NR_4$ ;

B represents  $CR_5$ ,  $NR_5$  or N;

D represents  $CR_6$ ,  $NR_6$  or N;

E represents  $CR_7$ ,  $NR_7$  or N;

10 with the condition that the ring containing substituent A has a maximum of two nitrogen atoms in its structure;

m, n and p represent: 0 or 1, where  $m + n + p = 2$  or 3;

15 the dashed lines - - - represent a single or double bond;

$R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$  and  $R_7$  each independently represent a radioactive isotope, H, a halogen or a radical optionally having a radioactive isotope, said radical being chosen from:  $C_1$ - $C_6$  alkyl, OH,  $C_1$ - $C_6$  polyhydroxyalkyl,  $C_1$ - $C_6$  alkoxyl,  $C_1$ - $C_6$  alkoxyalkyl,  $(CH_2)_q$ -OR', wherein q is 1, 2 or 3,  $CF_3$ ,  $CH_2$ - $CH_2$ F,  $O$ - $CH_2$ - $CH_2$ F,  $CH_2$ - $CH_2$ - $CH_2$ F, CN,  $NO_2$ ,  $O(CO)R'$ , OR',  $SR'$ ,  $COOR'$  - $SO_3H$ ,  $(CH_2)_r$ - $CO_2R''$ ,  $(CH_2)_r$ -CO-R', wherein r is 1, 2 or 3 and Rph, wherein Rph represents a non substituted or substituted phenol group, the possible substituents of the phenol group being any of the meanings of  $R_1$ - $R_7$  except for a phenol group;

30  $R'$  is H or a  $C_1$ -3 alkyl group;

$R''$  is H, a  $C_1$ - $C_6$  alkyl group or a  $C_1$ - $C_6$  alkyloxy group;

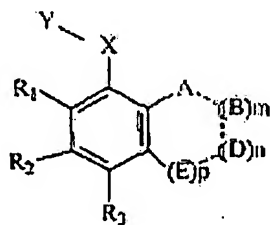
35 with the condition that only one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X, Y or Z is or has a radioactive isotope; in the preparation of a composition for diagnosis and/or monitoring

of diseases associated with the formation of amyloid protein fibrils, particularly those that appear as amyloid plaques and affect the central nervous system.

Within the context of the present invention, diseases associated with the formation of amyloid protein fibrils, particularly those that appear as amyloid plaques and affect the central nervous system, are understood to be diseases such as Alzheimer's, Parkinson's, Huntington, Creutzfeld-Jacob, cystic fibrosis, late onset diabetes, motor neuron disease, Mediterranean fever, Muckle-Wells syndrome, idiopathic myeloma, amyloid polyneuropathy, amyloid cardiomyopathy, senile systemic amyloidosis, hereditary cerebral haemorrhage with amyloidosis, Down syndrome, Creutzfeld-Jacob disease, Kuru, Gerstmann-Straussler-Schienenker syndrome, thyroid medullar carcinoma, amyloid valve deposits, amyloidosis in dialysis patients, inclusion body myositis, amyloid muscular deposits, Sickle Cell Parkinson anaemia, type 2 diabetes, amongst others.

A fourth aspect of the invention refers to compounds having the following structures:

a) Compounds of General Formula I



Formula I

wherein:

X represents O, S;

Y represents H or, along with X, where X = O, a carbohydrate radical;

A represents N or NR<sub>4</sub>;

B represents CR<sub>5</sub>, NR<sub>5</sub> or N;

D represents  $CR_6$ ,  $NR_6$  or N;

E represents  $CR_7$ ,  $NR_7$  or N;

with the condition that the ring containing group A has a maximum of two nitrogen atoms in its structure;

m, n and p represent: 0 or 1, where  $m + n + p = 2$  or 3;

the dashed lines - - - represent a single or double bond;

$R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$  and  $R_7$  each independently represent a radioactive isotope, H, a halogen or a radical optionally having a radioactive isotope, said radical being chosen from:  $C_1$ - $C_6$  alkyl, OH,  $C_1$ - $C_6$  polyhydroxyalkyl,  $C_1$ - $C_6$  alkoxyl,  $C_1$ - $C_6$  alkoxyalkyl,  $(CH_2)_q$ -OR', wherein q is 1, 2 or 3,  $CF_3$ ,  $CH_2$ - $CH_2$ F, O- $CH_2$ - $CH_2$ F,  $CH_2$ - $CH_2$ - $CH_2$ F, CN,  $NO_2$ , O(CO)R', OR', SR', COOR' - $SO_3H$ ,  $(CH_2)_r$ -CO $_2$ R'',  $(CH_2)_r$ -CO-R', wherein r is 1, 2 or 3 and Rph, wherein Rph represents a non substituted or substituted phenol group, the possible substituents of the phenol group being any of the meanings of  $R_1$ - $R_7$  except for a phenol group;

R' is H or a  $C_{1-3}$  alkyl group;

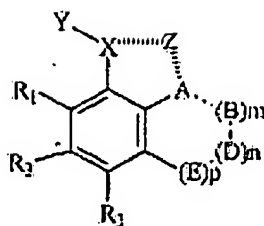
R'' is H, a  $C_1$ - $C_6$  alkyl group or a  $C_1$ - $C_6$  alkyloxy group;

with the condition that  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X and Y are not all simultaneously H, and

with the condition that only one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X and Y is or has a radioactive isotope;

Within the Formula I compounds, those satisfying the ratio  $m + n + p = 3$  are preferred.

b) Compounds of General Formula II



Formula II.

wherein:

X represents O, S;

Y represents H or, along with X, where X = O, a carbohydrate radical;

Z represents a metal or rare earth cation that may or may not be radioactive;

the ||||| line represents a coordinate bond;

A represents N or NR<sub>4</sub>;

B represents CR<sub>5</sub>, NR<sub>5</sub> or N;

D represents CR<sub>6</sub>, NR<sub>6</sub> or N;

E represents CR<sub>7</sub>, NR<sub>7</sub> or N;

with the condition that the ring containing group A has a maximum of two nitrogen atoms in its structure;

m, n and p represent: 0 or 1, where m + n + p = 2 or 3;

the dashed lines - - - represent a single or double bond;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub> and R<sub>7</sub> each independently represent a radioactive isotope, H, a halogen or a radical optionally having a radioactive isotope, said radical being chosen from: C<sub>1</sub>-C<sub>6</sub> alkyl, OH, C<sub>1</sub>-C<sub>6</sub> polyhydroxyalkyl, C<sub>1</sub>-C<sub>6</sub> alkoxyl, C<sub>1</sub>-C<sub>6</sub> alkoxyalkyl, (CH<sub>2</sub>)<sub>q</sub>-OR', wherein q is 1, 2 or 3, CF<sub>3</sub>, CH<sub>2</sub>-CH<sub>2</sub>F, O-CH<sub>2</sub>-CH<sub>2</sub>F, CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>F, CN, NO<sub>2</sub>, O(CO)R', OR', SR', COOR' -SO<sub>3</sub>H, (CH<sub>2</sub>)<sub>r</sub>-CO<sub>2</sub>R'', (CH<sub>2</sub>)<sub>r</sub>-CO-R', wherein r is 1, 2 or 3 and R<sub>ph</sub>,

wherein Rph represents a non substituted or substituted phenol group, the possible substituents of the phenol group being any of the meanings of  $R_1$ - $R_7$  except for a phenol group;

5

$R'$  is H or a  $C_{1-3}$  alkyl group;

$R''$  is H, a  $C_1$ - $C_6$  alkyl group or a  $C_1$ - $C_6$  alkyloxy group;

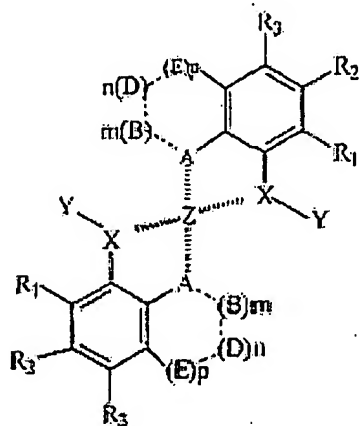
with the condition that  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X and Y are not all simultaneously H, and

10

with the condition that only one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X, Y or Z is or has a radioactive isotope;

and, finally,

c) Compounds of General Formula III



Formula III

15

wherein:

X represents O, S;

Y represents H or, along with X, where  $X = O$ , a carbohydrate radical;

20

Z represents a metal or rare earth cation that may or may not be radioactive;

the ||||| line represents a coordinate bond;

A represents N or  $NR_4$ ;

B represents  $CR_5$ ,  $NR_5$  or N;

25

D represents  $CR_6$ ,  $NR_6$  or N;

E represents  $CR_7$ ,  $NR_7$  or N;

with the condition that the ring containing group A has a maximum of two nitrogen atoms in its structure;

5 m, n and p represent: 0 or 1, where  $m + n + p = 2$  or 3;

the dashed lines - - - represent a single or double bond;

10  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$  and  $R_7$  each independently represent a radioactive isotope, H, a halogen or a radical optionally having a radioactive isotope, said radical being chosen from:  $C_1$ - $C_6$  alkyl, OH,  $C_1$ - $C_6$  polyhydroxyalkyl,  $C_1$ - $C_6$  alkoxyl,  $C_1$ - $C_6$  alkoxyalkyl,  $(CH_2)_q$ -OR', wherein q is 1, 2 or 3, 15  $CF_3$ ,  $CH_2$ - $CH_2$ F, O- $CH_2$ - $CH_2$ F,  $CH_2$ - $CH_2$ - $CH_2$ F, CN,  $NO_2$ , O(CO)R', OR', SR', COOR' -SO<sub>3</sub>H,  $(CH_2)_r$ -CO<sub>2</sub>R'',  $(CH_2)_r$ -CO-R', wherein r is 1, 2 or 3 and Rph, wherein Rph represents a non substituted or substituted phenol group, the possible substituents of the phenol group being any of the meanings of  $R_1$ - $R_7$  except for a phenol group;

R' is H or a  $C_{1-3}$  alkyl group;

R'' is H, a  $C_1$ - $C_6$  alkyl group or a  $C_1$ - $C_6$  alkyloxy group;

25 with the condition that  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X and Y are not all simultaneously H, and

with the condition that only one of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , X, Y or Z is or has a radioactive isotope;

and with the condition that when

30 A is N,

B, D and E are all CH,

X is O, and

m, n and p are all 1,

then  $R_1$ ,  $R_2$  and  $R_3$  are not all H.

35

Preferred compounds for use in the diagnosis and monitoring of diseases associated with the formation of amyloid protein fibrils are chosen from the following:

5 a) Compounds according to General Formula I:

Analogues with radioactive iodine.

5-chloro-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline  
 5-chloro-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline  
 10 5-[<sup>123</sup>I]iodo-7-iodo-8-hydroxyquinoline  
 5-iodo-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline  
 5-[<sup>124</sup>I]iodo-7-iodo-8-hydroxyquinoline  
 5-iodo-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline

15 Analogues with radioactive fluorine.

5-chloro-7-[<sup>18</sup>F]fluoro-8-hydroxyquinoline  
 5-[<sup>18</sup>F]fluoro-7-iodo-8-hydroxyquinoline

Analogues with radioactive carbon.

20 5-chloro-7-iodo-8-[<sup>11</sup>C]methoxyquinoline

Glucuronides.

5-chloro-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline glucuronide  
 5-chloro-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline glucuronide  
 25 5-chloro-7-[<sup>18</sup>F]fluoro-8-hydroxyquinoline glucuronide  
 5-[<sup>18</sup>F]fluoro-7-iodo-8-hydroxyquinoline glucuronide  
 5-chloro-7-iodo-8-[<sup>11</sup>C]methoxyquinoline glucuronide

Analogues with a single halogen atom.

30 5-[<sup>123</sup>I]-8-hydroxyquinoline  
 5-[<sup>124</sup>I]-8-hydroxyquinoline  
 7-[<sup>123</sup>I]-8-hydroxyquinoline  
 7-[<sup>124</sup>I]-8-hydroxyquinoline  
 5-[<sup>18</sup>F]-8-hydroxyquinoline  
 35 5-[<sup>18</sup>F]-8-hydroxyquinoline



## b) Compounds according to General Formula II:

5	<u>Metal complexes with 5-chloro-7-<sup>[123]I</sup>iodo-8-hydroxyquinoline</u>
	5-chloro-7- <sup>[123]I</sup> iodo-8-hydroxyquinoline Fe(II) complex
	5-chloro-7- <sup>[123]I</sup> iodo-8-hydroxyquinoline Cu(II) complex
	5-chloro-7- <sup>[123]I</sup> iodo-8-hydroxyquinoline Zn(II) complex
	5-chloro-7- <sup>[123]I</sup> iodo-8-hydroxyquinoline Mn(II) complex
10	<u>Metal complexes with 5-chloro-7-<sup>[124]I</sup>iodo-8-hydroxyquinoline</u>
	5-chloro-7- <sup>[124]I</sup> iodo-8-hydroxyquinoline Fe(II) complex
	5-chloro-7- <sup>[124]I</sup> iodo-8-hydroxyquinoline Cu(II) complex
15	5-chloro-7- <sup>[124]I</sup> iodo-8-hydroxyquinoline Zn(II) complex
	5-chloro-7- <sup>[124]I</sup> iodo-8-hydroxyquinoline Mn(II) complex
	<u>Metal complexes with 5-chloro-7-<sup>[18]F</sup>fluoro-8-hydroxyquinoline</u>
20	5-chloro-7- <sup>[18]F</sup> fluoro-8-hydroxyquinoline Fe(II) complex
	5-chloro-7- <sup>[18]F</sup> fluoro-8-hydroxyquinoline Cu(II) complex
	5-chloro-7- <sup>[18]F</sup> fluoro-8-hydroxyquinoline Zn(II) complex
	5-chloro-7- <sup>[18]F</sup> fluoro-8-hydroxyquinoline Mn(II) complex
25	<u>Metal complexes with 5-<sup>[18]F</sup>fluoro-7-iodo-8-hydroxyquinoline</u>
	5- <sup>[18]F</sup> fluoro-7-iodo-8-hydroxyquinoline Fe(II) complex
	5- <sup>[18]F</sup> fluoro-7-iodo-8-hydroxyquinoline Cu(II) complex
	5- <sup>[18]F</sup> fluoro-7-iodo-8-hydroxyquinoline Zn(II) complex
30	5- <sup>[18]F</sup> fluoro-7-iodo-8-hydroxyquinoline Mn(II) complex
	<u>Metal complexes with 5-chloro-7-iodo-8-<sup>[11]C</sup>methoxyquinoline</u>
	5-chloro-7-iodo-8- <sup>[11]C</sup> methoxyquinoline Fe(II) complex
35	5-chloro-7-iodo-8- <sup>[11]C</sup> methoxyquinoline Cu(II) complex

5-chloro-7-iodo-8-[<sup>11</sup>C]methoxyquinoline Zn(II) complex

5-chloro-7-iodo-8-[<sup>11</sup>C]methoxyquinoline Mn(II) complex

Metal complexes with 5-chloro-7-iodo-8-hydroxyquinoline

5 5-chloro-7-iodo-8-hydroxyquinoline <sup>99m</sup>Tc complex

5-chloro-7-iodo-8-hydroxyquinoline <sup>111</sup>In complex

5-chloro-7-iodo-8-hydroxyquinoline <sup>201</sup>Tl complex

5-chloro-7-iodo-8-hydroxyquinoline <sup>67</sup>Ga complex

5-chloro-7-iodo-8-hydroxyquinoline <sup>68</sup>Ga complex

10 5-chloro-7-iodo-8-hydroxyquinoline <sup>67</sup>Cu complex

5-chloro-7-iodo-8-hydroxyquinoline <sup>64</sup>Cu complex

c) Compounds according to General Formula III:

15 Bis-chelate metal complexes with 5-chloro-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline

5-chloro-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline Fe(II) bis-chelate complex

20 5-chloro-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline Cu(II) bis-chelate complex

5-chloro-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline Zn(II) bis-chelate complex

5-chloro-7-[<sup>123</sup>I]iodo-8-hydroxyquinoline Mn(II) bis-chelate complex

25 Bis-chelate metal complexes with 5-chloro-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline

5-chloro-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline Fe(II) bis-chelate complex

30 5-chloro-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline Cu(II) bis-chelate complex

5-chloro-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline Zn(II) bis-chelate complex

35 5-chloro-7-[<sup>124</sup>I]iodo-8-hydroxyquinoline Mn(II) bis-chelate complex

Bis-chelate metal complexes with 5-chloro-7-<sup>18</sup>F-fluoro-8-hydroxyquinoline

5	5-chloro-7- <sup>18</sup> F-fluoro-8-hydroxyquinoline	Fe(II)	bis-chelate complex
	5-chloro-7- <sup>18</sup> F-fluoro-8-hydroxyquinoline	Cu(II)	bis-chelate complex
	5-chloro-7- <sup>18</sup> F-fluoro-8-hydroxyquinoline	Zn(II)	bis-chelate complex
10	5-chloro-7- <sup>18</sup> F-fluoro-8-hydroxyquinoline	Mn(II)	bis-chelate complex

Bis-chelate metal complexes with 5-<sup>18</sup>F-fluoro-7-iodo-8-hydroxyquinoline

15	5- <sup>18</sup> F-fluoro-7-iodo-8-hydroxyquinoline	Fe(II)	bis-chelate complex
	5- <sup>18</sup> F-fluoro-7-iodo-8-hydroxyquinoline	Cu(II)	bis-chelate complex
	5- <sup>18</sup> F-fluoro-7-iodo-8-hydroxyquinoline	Zn(II)	bis-chelate complex
20	5- <sup>18</sup> F-fluoro-7-iodo-8-hydroxyquinoline	Mn(II)	bis-chelate complex

Bis-chelate metal complexes with 5-chloro-7-iodo-8-<sup>11</sup>C-methoxyquinoline

25	5-chloro-7-iodo-8- <sup>11</sup> C-methoxyquinoline	Fe(II)	bis-chelate complex
	5-chloro-7-iodo-8- <sup>11</sup> C-methoxyquinoline	Cu(II)	bis-chelate complex
30	5-chloro-7-iodo-8- <sup>11</sup> C-methoxyquinoline	Zn(II)	bis-chelate complex
	5-chloro-7-iodo-8- <sup>11</sup> C-methoxyquinoline	Mn(II)	bis-chelate complex

Bis-chelate metal complexes with 5-chloro-7-iodo-8-

hydroxyquinoline

	5-chloro-7-iodo-8-hydroxyquinoline complex	<sup>99m</sup> Tc	bis-chelate
5	5-chloro-7-iodo-8-hydroxyquinoline complex	<sup>111</sup> In	bis-chelate
	5-chloro-7-iodo-8-hydroxyquinoline complex	<sup>201</sup> Tl	bis-chelate
	5-chloro-7-iodo-8-hydroxyquinoline complex	<sup>67</sup> Ga	bis-chelate
10	5-chloro-7-iodo-8-hydroxyquinoline complex	<sup>68</sup> Ga	bis-chelate
	5-chloro-7-iodo-8-hydroxyquinoline complex	<sup>67</sup> Cu	bis-chelate
15	5-chloro-7-iodo-8-hydroxyquinoline complex	<sup>64</sup> Cu	bis-chelate

The compounds of the present invention are prepared according to methods known in the state of the art. Thus:

The compounds of Formula I are prepared according to a method comprising making a quinoline derivative react:

- a) with an electrophilic aromatic halogenation reagent incorporating a radioactive halogen atom, or;
- b) with a radioactive halogenated derivative to effect an aromatic nucleophilic substitution reaction.

The compounds of Formula II are prepared according to a method comprising making a quinoline derivative react:

- a) with a metal or rare earth cation, or,
  - b) with a radioactive isotope of these elements,
- such that the metal or rare earth cation or the radioactive isotope of these elements is in a suitable oxidation state so as to produce the corresponding chelating product of Formula II.

The compounds of Formula III are prepared according to conventional methods known in the state of the art. The preferred methods comprise making a quinoline derivative react

with:

c) a metal or rare earth cation, or,

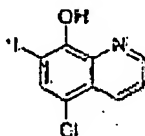
d) a radioactive isotope of these elements

in a suitable oxidation state so as to produce the  
corresponding chelating product defined in Formula III.

#### Preparative Examples

##### EXAMPLE 1:

Preparation of 5-chloro-8-hydroxy-7- $^{125}$ I}iodoquinoline by  
isotope exchange.



A 1-3 mCi solution of  $\text{Na}^{125}\text{I}$  is introduced in a 10 mL  
balloon and is evaporated to dryness at  $100^\circ\text{C}$  under a nitrogen  
current. The corresponding 7-iodoquinoline is added (100 mg)  
dissolved in 2 mL of a suitable solvent. The balloon is joined  
to a reflux condenser and the bath temperature is raised. The  
reaction mixture is stirred under nitrogen atmosphere for a  
certain time and is let to cool. Water is added and the  
product that is collected by filtration is rinsed well with  
water. It is recrystallised and the purity is determined by  
means of thin layer radiochromatography.

##### EXAMPLE 2:

Preparation of 5-chloro-8-hydroxy-7-trimethylstannyl-  
quinoline.

Hexamethylditin (1.31 mmol) is added to an 0.88 mmol  
mixture of iodine derivative (5-chloro-8-hydroxy-7-  
iodoquinoline) and tetrakis-triphenylphosphine palladium(0)  
(0.05 g) in 12 mL of 1,4-dioxane and the mixture is heated  
under reflux under nitrogen atmosphere for 6.5 hours. After  
cooling, the crude reaction product is filtered and the  
insoluble material is washed with ethyl acetate. The solvent

is eliminated under reduced pressure, obtaining a yellowish oil which is subject to silica gel column chromatography to produce a solid with a 65% yield.

5

## EXAMPLE 3:

Synthesis of 5-chloro-8-hydroxy-7-[<sup>123</sup>I]iodoquinoline from the organotin derivative using chloramine T.

[<sup>123</sup>I]sodium iodide (2-20 mCi) is added to a solution of the trimethyltin derivative described in Example 2 (200-300 ug) in 300 uL of ethanol, followed by chloramine T (100 ug) in 1N HCl (100 uL). After stirring for 5 minutes the reaction is stopped with an aqueous solution of metabisulphite (50 mg/mL, 100 uL) and is injected in the RP-HPLC. The radioiodinated derivative fraction obtained is collected with a 98% radiochemical purity.

15

## EXAMPLE 4:

Synthesis of 5-chloro-8-hydroxy-7-[<sup>131</sup>I]iodoquinoline from the organotin derivative using hydrogen peroxide.

20

The radioiodinated derivative is obtained from the tributyltin derivative prepared in an analogous manner to the organotin derivative described in example 2 by suspension in methanol and treatment with Na<sup>131</sup>I and hydrogen peroxide at room temperature, as described below. The reaction takes place by adding 50 uL of H<sub>2</sub>O<sub>2</sub> (3% w/v) to a mixture formed by 50 uL of the tributyltin derivative (100 ug/50 uL EtOH), 1.5 mCi of [<sup>125</sup>I]sodium iodide (specific activity 2200 Ci/mmol) and 100 uL of 1N HCl in a sealed vial. The reaction is stirred at room temperature for 10 minutes and is finalised after adding 100 uL of a saturated solution of anhydrous Na<sub>2</sub>SO<sub>3</sub> and the solvent is evaporated by means of a nitrogen current. It is neutralized with sodium bicarbonate and extracted with ethyl acetate. The extract is dried by passing the solution over a column of anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent is evaporated by means of nitrogen current. The crude reaction product is

30

35

purified by HPLC obtaining a radiochemical purity over 85%. The fractions are dried and the residue is extracted again with EtOAc. The different EtOAc extracts are concentrated under nitrogen atmosphere until dry. The residue is dissolved in EtOH. The radiochemical purity of the final product exceeds 98% (by HPLC).

EXAMPLE 5:

Synthesis of 5-chloro-8-hydroxy-7-[<sup>131</sup>I]iodoquinoline using chloramine T.

1.0 mL of an 0.02 M KH<sub>2</sub>PO<sub>4</sub> buffer solution (pH 4.8) is added to a vial containing 5-chloro-8-hydroxyquinoline. The mixture is heated to 40°C and is stirred to obtain a transparent solution which is cooled to room temperature. 10 mL of an 0.1 N NaOH solution containing 10.0 mCi of [<sup>131</sup>I]sodium iodide is added and the vial is closed with a Teflon stopper. A 7.5 ug chloramine T (N-chloro-p-toluenesulphonamide sodium salt) solution in 30 uL of the 0.02 M KH<sub>2</sub>PO<sub>4</sub> buffer solution is added. It is mechanically stirred at room temperature for 5 minutes and then manually for some seconds. After continuing stirring for 5 more minutes an aqueous solution of NaHSO<sub>3</sub> (1.4 mg/mL) is added and the reaction mixture is analysed by thin layer chromatography. The solution is passed over an anionic exchange column to eliminate free radioiodine.

EXAMPLE 6:

Synthesis of 5-chloro-8-hydroxy-7-[<sup>125</sup>I]iodoquinoline using chloramine T.

A 30 nmol solution of 5-chloro-8-hydroxyquinoline in 30 uL of ethanol is added to 10 mCi of [<sup>125</sup>I]sodium iodide (4.5 ug, 330 Ci/mmol, 30 nmol) in 0.001 N NaOH (30 uL). A solution of chloramine T (13 ug, 50 nmol) in water (10 uL) is added. The mixture is heated to 60°C during 45 minutes. 100 uL of 0.1 N NH<sub>4</sub>Cl are added and it is extracted with ether. The reaction

result is analysed by thin layer chromatography (Merck F254 Silica gel) in the appropriate eluent and then treated as in the previous example.

5

## EXAMPLE 7:

Synthesis of 5-chloro-8-hydroxy-7-<sup>[131]I</sup>iodoquinoline using Iodogen™.

10

100 uL of a Iodogen solution (1,3,4,6-tetrachloro-3-  
alpha,6-alpha-diphenylglycoluril) (1.0 mg/mL in CH<sub>2</sub>Cl<sub>2</sub>) are  
added to a 5 mL vial. Dichloromethane evaporates from the vial  
due to nitrogen current. A 5-chloro-8-hydroxyquinoline  
solution (0.5 mg/0.5 mL) in 0.02M KH<sub>2</sub>PO<sub>4</sub>, pH 4.8 is added to  
the vial, which is then closed with a Teflon stopper and a  
solution containing approximately 10 mCi of <sup>[131]I</sup>sodium iodide  
is introduced by means of a syringe. The mixture is stirred at  
room temperature for 30 minutes. A radiochemical purity  
exceeding 99% is observed by thin layer chromatography in  
silica gel using suitable eluents.

15

20

## EXAMPLE 8:

Preparation of 5-chloro-8-hydroxy-7-<sup>[125]I</sup>iodoquinoline using Iodo-beads.

25

The Iodo-beads are previously washed with a 0.1 M sodium  
phosphate buffer solution at pH = 6.5. The necessary amount of  
Iodo-beads is added to an Na<sup>125</sup>I solution in buffer solution at  
a concentration of 1 mCi for each 100 ug of product to be  
iodinated. Between 5 and 500 ug of the phenol derivative  
dissolved in the same buffer solution are then added thereto.  
It is left to react between 2 and 15 minutes at room  
temperature. The reaction is stopped by separating the Iodo-  
beads from the solution by decantation. The Iodo-beads are  
washed with the buffer solution in order to recover all the  
radioiodinated derivative.

30

35

## EXAMPLE 9:



Synthesis of 5-chloro-8-hydroxy-7-[<sup>123</sup>I]iodoquinoline using peracetic acid.

0.19 umol of 5-chloro-8-hydroxyquinoline precursor dissolved in 50 uL of ethanol are mixed in a vial with a known volume of a buffered solution at pH 2 which is twice the volume of the basic commercial [<sup>123</sup>I]iodine solution. This solution is introduced in the vial containing the radioactive iodine, followed by 50 uL of a 3.2% solution of peracetic acid (obtained from a stock solution at 32% w/v). The sealed vial is heated to 65°C for 12 minutes. It is left to cool and an aqueous solution of NaHSO<sub>3</sub> is added. The radiochemical yield is purified and determined by chromatographic methods such as those described in previous examples.

EXAMPLE 10:

Preparation of 5-chloro-7-[<sup>18</sup>F]fluoro-hydroxyquinoline by direct radiofluorination.

Direct radiofluorination of 100 umol of the phenol derivative 5-chloro-8-hydroxyquinoline is performed by dissolving the compound in a mixture of trifluoroacetic acid and glacial acetic acid (1:1) through which freshly prepared acetyl hypofluorite ([<sup>18</sup>F]CH<sub>3</sub>-COOF) is bubbled. The solvent is evaporated and the residue is purified by means of HPLC.

EXAMPLE 11:

Synthesis of 5-[<sup>18</sup>F]fluoro-hydroxyquinoline from 5-fluoro-8-hydroxyquinoline by isotope exchange.

The radioactive fluoride [<sup>18</sup>F] is transferred to a 5 mL borosilicate reaction vial and is azeotropically dried with acetonitrile in the presence of 4.0 mg of K<sub>2</sub>CO<sub>3</sub> and 14.6 mg of Kryptofix 2.2.2®. The precursor fluoride derivative dissolved in 0.5 mL of DMSO is added to the vial containing the dry radioactive fluoride, the K<sub>2</sub>CO<sub>3</sub> and the Kryptofix 2.2.2® and the isotope exchange reaction is performed heating to 110, 130 and 160°C for 0-30 minutes to optimise the reaction.

Purification of the radiofluorinated derivative is performed by reverse phase HPLC. The corresponding fractions are collected and concentrated under reduced pressure.

5

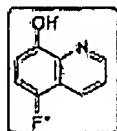
**EXAMPLE 12:**Preparation of 5-chloro-8-hydroxy-7- $^{123}\text{I}$ iodoquinoline from 5-chloro-7-fluoro-8-hydroxyquinoline.

10

15

The starting material dissolved in 600  $\mu\text{L}$  of ethanol is added to 100  $\mu\text{L}$  of a solution A (containing 200  $\mu\text{mol}$  of  $\text{SnSO}_4$ , 2 mmol of gentisic acid, 2 mmol of monohydrate citric acid, 10  $\mu\text{L}$  of glacial acetic acid dissolved in 10% acetic acid), 25  $\mu\text{L}$  of a solution B (containing 400  $\mu\text{mol}$  of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  dissolved in 10 mL of water) and 65  $\mu\text{L}$  of glacial acetic acid. The vial is placed in an ultrasound bath for 15 minutes, and 10  $\mu\text{L}$  of  $\text{Na}^{123}\text{I}$  (about 1 mCi) are added.  $\text{N}_2$  is passed through the mixture and it is heated to  $60^\circ\text{C}$  for 1 hour. It is left to cool at room temperature and the crude reaction product is purified by means of HPLC.

20

**EXAMPLE 13:**Preparation of 5- $^{18}\text{F}$ fluoro-hydroxyquinoline by aromatic nucleophilic substitution.

25

The corresponding halo- or nitro- derivative is made to react with the fluorine-18 ion by aromatic nucleophilic substitution using the  $^{18}\text{F}$ FK-K222 complex in DMSO and

30

heating to  $150\text{--}180^\circ\text{C}$  in the conventional manner for 10 minutes or activating the reaction mixture with a 100 Watt microwave during 1-2.5 minutes. The characterisation and isolation of the radiolabelled product is performed as in Example 11.

## EXAMPLE 14:

Synthesis of 5-chloro-7-[<sup>18</sup>F]fluoro-8-hydroxyquinoline from the deprotected precursor.

5           The electrophilic radiofluorination agent [<sup>18</sup>F]CH<sub>3</sub>COOF, prepared from 100 umol [<sup>18</sup>F]F<sub>2</sub>, or [<sup>18</sup>F]OF<sub>2</sub> (100 umol), is made to bubble for 10 minutes at room temperature in a solution (101 umol) of the trimethyltin derivative 5-chloro-8-hydroxy-7-trimethylstannyl-quinoline or 5-chloro-8-hydroxy-7-tributylstannyl-quinoline in 10 mL of Freon-11 (CFCl<sub>3</sub>). The solvent is evaporated at 50°C with a nitrogen current and the residue is dissolved in 10 mL of methylene chloride and is transferred to a chromatographic column packed with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (2.5 cm) and silica gel (9.5 cm) which has previously been equilibrated with ether. It is eluted with ether. The solvent is evaporated and the solution is purified by means of semi-preparative HPLC.

## EXAMPLE 15:

Synthesis of 5-chloro-7-[<sup>18</sup>F]fluoro-8-hydroxyquinoline from the protected precursor.

20           The electrophilic radiofluorination agent [<sup>18</sup>F]CH<sub>3</sub>COOF, prepared from 100 umol [<sup>18</sup>F]F<sub>2</sub>, or [<sup>18</sup>F]OF<sub>2</sub> (100 umol), is made to bubble for 10 minutes at room temperature in a solution (101 umol) of the trimethyltin derivative 5-chloro-8-t-butoxycarbonyloxy-7-trimethylstannyl-quinoline or 5-chloro-8-t-butoxycarbonyloxy-7-tributylstannyl-quinoline in 10 mL of Freon-11 (CFCl<sub>3</sub>). The solvent is evaporated at 50°C with a nitrogen current and the residue is dissolved in 10 mL of methylene chloride and is transferred to a chromatographic column packed with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (2.5 cm) and silica gel (9.5 cm) which has previously been equilibrated with ether. It is eluted with ether. The solvent is evaporated and the derivative is hydrolysed in the presence of a 48% hydrobromic acid solution at 130°C for 10 min. After cooling, the reaction

mixture is partially neutralised with a 3 N NaOH solution and purified by means of HPLC.

EXAMPLE 16:

5     Preparation of 5-chloro-7-[<sup>18</sup>F]fluoro-8-hydroxyquinoline by  
      substituting trimethylammonium with fluorine.

260 mg (1.5 equiv.) of HNMe<sub>2</sub>.HCl and 430 mg (1.5 equiv.)  
of K<sub>2</sub>CO<sub>3</sub> are added to a 2.1 mmol solution of the precursor  
fluorine derivative in a mixture of 30 mL of DMSO and 10 mL of  
10     water at 10°C. After stirring at 10°C for 10 minutes, the  
solution is heated under reflux for 24 hours and left to cool,  
diluted with water (50 mL) and the product is extracted with  
ether. After processing the reaction, the residue is purified  
by silica gel chromatography. In a second reaction step the  
15     following process is followed: 1.45 equiv. of methyl  
trifluoromethanesulphonate are added to a solution of 0.65  
mmol of dimethyl amino derivative in 2 mL of toluene. The  
solution is stirred for 1 hour at room temperature under  
nitrogen atmosphere and is then diluted with 100 mL of water.  
20     The product is extracted with dichloromethane, the solvent is  
evaporated and the remaining product is ground with diethyl  
ether. In a final stage, about 2.0-3.5 mg (6.5-11.3 umol) of  
the methyl trifluoromethanesulphonate derivative are dissolved  
in 600 uL of freshly distilled DMSO and added directly to the  
25     tube containing the K[<sup>18</sup>F]F-K222 anhydrous complex. The tube  
is heated on a heating block at 145-150°C without stirring for  
2 minutes or is placed in a 100 W microwave oven for 1 minute.  
The reaction mixture is left to cool and is diluted with 3 mL  
of water and filtered through a C18 Sep Pack cartridge. The  
30     cartridge is washed with 3.0 mL of water and partially dried  
for 0.5 minutes applying nitrogen current. The  
radiofluorinated derivative is eluted from the cartridge with  
DCM (3 mL) followed by two more washings each of 1 mL.

35     EXAMPLE 17:

Synthesis of the clioquinol glycoconjugate, 5-chloro-8-yl-iodoquinoline-beta-D-glucuronic acid sodium salt.

A mixture of 5-chloro-8-hydroxy-7-iodoquinoline (50 mg, 0.164 mmol), methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-D-glucopyranoside uronate (65 mg, 0.164 mmol),  $\text{CaSO}_4 \cdot \text{H}_2\text{O}$  (35 mg) in pyridine (1.5 mL) is stirred at room temperature for a few minutes.  $\text{Ag}_2\text{CO}_3$  (35 mg) is added to the reaction and the suspension is stirred at room temperature for 20 hours, protected from light. Once the reaction has finished and after isolating the product, the deprotection of the hydroxyl protecting groups is performed using a 1 N NaOH solution. The reaction is diluted with dichloromethane, filtered and the solvent is removed at reduced pressure. The glycoconjugate is purified by means of flash column chromatography. (TLC:  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  99/1, eluent:  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  99.5/0.5). NMR (400 MHz,  $\text{CDCl}_3$ ) 2.04 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.13 (s, 3H, Ac), 3.68 (s, 3H, Me), 3.99 (d, 1H, 5'H), 5.40-5.52 (m, 3H, 2', -3', -4'-H), 6.29 (d, 1H, 1'-H), 7.56 (m, 1H, 3H), 7.99 (s, 1H, 6-H), 8.52 (d, 1H, 4-H), 8.93 (s, 1H, 2-H).

EXAMPLE 18:

Radioiodination of the clioquinol glucuronide with Iodogen™:  
Synthesis of the 5-chloro-8-hydroxy-7- $^{131}\text{I}$ iodoquinoline glucuronide.

10 mg of Iodogen™ are dissolved in 2 mL of dichloromethane in a tube containing small crystals in order to increase contact of the solution with the Iodogen™ (1,3,4,6-tetrachloro-3 $\alpha$ ,6 $\alpha$ -diphenylglycoluril). The solvent is evaporated and a white film is observed inside the tube and on the small crystals. 1 mL of an aqueous solution of the corresponding glucuronide (4 mg/mL) is added and then  $7.4 \times 10^7$  Bq (2 mCi) of  $\text{Na}^{131}\text{I}$  is added. The solution is kept at room temperature while stirring for 10 minutes. The reaction is followed by TLC and the  $R_f$  of the radiochromatography-labelled product is determined. It can be observed that a single

radioiodinated product has been obtained with a radioactive yield between 90 and 95%.

EXAMPLE 19:

5        Radioiodination of the clioquinol glucuronide with  
chloramine T: Synthesis of the 5-chloro-8-hydroxy-7-  
10        [<sup>125</sup>I]iodoquinoline glucuronide.

A 4 mg/mL solution of chloramine T in 50 mM of disodium phosphate at pH 7 is prepared. 25 uL of a standard solution of the glycoconjugate in 50 uM of sodium phosphate at pH 7 is added to 2 uL of a 0.5 mCi/uL Na<sup>125</sup>I solution. The vial is closed and 25 uL of the chloramine T solution are added, this mixture is stirred for 30 seconds. Once the reaction has finished 100 uL of 12.6 mM sodium metabisulphite are added. It is stirred for 10 seconds. The reaction product is purified by filtration on gel using a Sephadex G-25 or G-50 column.

EXAMPLE 20:

20        Radioiodination of the clioquinol glucuronide with Iodo-  
beads: Synthesis of the 5-chloro-8-hydroxy-7-  
      [<sup>125</sup>I]iodoquinoline glucuronide.

25        25 uL of a solution of the glycoconjugate in 50 uM of sodium phosphate at pH 7 are added to 2 uL of a 0.5 mCi/uL Na<sup>125</sup>I solution. The vial is closed and some Iodo-beads are added and it is stirred for 30 seconds. Once the reaction is finished the polymer is separated from the supernatant. 100 uL of 12.6 mM sodium metabisulphite are added to this solution. It is stirred for 10 seconds. The reaction product is purified by filtration on gel using a Sephadex G-25 or G-50 column.

EXAMPLE 21:

Synthesis of the 5,7-dichloro-8-hydroxyquinoline  
35        [<sup>111</sup>In]indium complex.

The trihydrate [<sup>111</sup>In]indium chloride (<sup>111</sup>InCl<sub>3</sub>.3H<sub>2</sub>O, 1.37 g, 5.0 mmol) and the 5,7-dichloro-8-hydroxy-quinoline (2.14 g,

10 mmol) are dissolved in ethanol (200 mL) and the solution is concentrated to dryness at reduced pressure. The yellowish crude product obtained is recrystallised from ethanol after adding water to give the 5,7-dichloro-8-hydroxyquinoline [ $^{111}\text{In}$ ]indium complex.

EXAMPLE 22:

Synthesis of the 8-hydroxyquinoline [ $^{67}\text{Ga}$ ]gallium chelator.

The [ $^{67}\text{Ga}$ ]gallium chelator is prepared by adding [ $^{67}\text{Ga}$ ]gallium trichloride ( $^{67}\text{GaCl}_3$ ) dissolved in an 0.05 M HCl solution to a 7 mM aqueous solution of 8-hydroxyquinoline at pH 3.5. After about 25 minutes of stirring the pH increases to 6. The extraction of the product with chloroform leads to the chelator, which is obtained with a 90% yield.

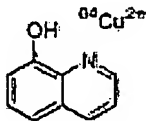
EXAMPLE 23:

Preparation of the 8-hydroxyquinoline [ $^{99\text{m}}\text{Tc}$ ]technetium chelator.

The 8-hydroxyquinoline (0.51 mmol) is dissolved in 3 mL of a 0.1 N NaOH solution and the solution pH is adjusted to pH = 3.5 with 1 N HCl. 0.3 mL of an  $\text{SnCl}_2$  solution (20 mg, 0.11 mmol in 10 mL of 1 N HCl) are added and the pH is again adjusted with 0.1 N NaOH. It is stirred for 5 minutes and 80  $\mu\text{Ci}$  of technetium-99m are added in the form of sodium pertechnetate. The chelator is obtained by extracting with chloroform with a yield exceeding 90%.

EXAMPLE 24:

Synthesis of the 8-hydroxyquinoline [ $^{64}\text{Cu}$ ]copper chelator.



A [ $^{64}\text{Cu}$ ] copper (II) chloride ( $^{64}\text{CuCl}_2$ ) solution is diluted 10 times with a 0.1 M ammonium acetate buffer solution of pH 5.5. The [ $^{64}\text{Cu}$ ] copper acetate is added to 8-hydroxyquinoline and the final volume is adjusted to 1.0-1.5 mL with the buffer solution. After incubating at room temperature for 45 minutes, the extraction of the  $^{64}\text{Cu}$  derivative product with chloroform leads to the chelator, which is obtained with a 90% yield.

#### EXAMPLE 25:

##### Synthesis of the 8-hydroxyquinoline [ $^{67}\text{Cu}$ ]copper chelator.

An aliquot of the starting solution ( $^{67}\text{Cu}^{2+}/\text{HCl}$ ) is evaporated to dryness under nitrogen current and reconstituted with an 0.25 N acetate buffered solution (pH = 5.5). The ligand (0.2 mg) is dissolved in DMSO (1 mg/10 uL) and is added to 50 uL of the [ $^{67}\text{Cu}$ ] copper acetate solution (50 uL). After adding 50 uL of ethanol, the [ $^{67}\text{Cu}$ ] copper derivative is filtered through a polytetrafluoroethylene (PTFE) membrane. The radiochemical purity is determined by thin layer chromatography in silica gel plaques eluting with ethanol.

#### EXAMPLE 26:

##### Synthesis of 5-chloro-8-hydroxy-7- $^{125}\text{I}$ iodoquinoline copper chelators.

##### METHOD A: Chelation of 5-chloro-8-hydroxy-7- $^{125}\text{I}$ iodoquinoline.

A copper (II) chloride solution is diluted 10 times with 0.1 M ammonium acetate of pH 5.5. This solution is added to 5-chloro-8-hydroxy-7- $^{125}\text{I}$ iodoquinoline and the final volume is adjusted to 1.0-1.5 mL with a 0.1 M ammonium acetate buffer solution at pH = 5.5. After incubating at room temperature for 45 minutes, the extraction of the solution with chloroform



leads to the chelator, which is obtained with a 90% yield.

METHOD B: Radioiodination of the 5-chloro-8-hydroxyquinoline copper chelator.

5 A 30 nmol solution of 5-chloro-8-hydroxyquinoline copper chelator in 30 uL of ethanol is added to 10 mCi of [<sup>125</sup>I]sodium iodide (4.5 ug, 330 Ci/mmol, 30 nmol) in 0.001 N NaOH (30 uL). A chloramine T solution (13 ug, 50 nmol) in water (10 uL) is added. The mixture is heated to 60°C for 45 minutes. 100 uL of  
10 0.1 N NH<sub>4</sub>Cl is added and extracted with ether. The product is analysed by thin layer chromatography (Silica gel, Merck F254) in the appropriate eluent.

EXAMPLE 27:

15 Synthesis of 5-chloro-8-hydroxy-7-<sup>125</sup>I]iodoquinoline zinc chelators.

METHOD A: Chelation of 5-chloro-8-hydroxy-7-<sup>125</sup>I]iodoquinoline.

20 Zn(OAc)<sub>2</sub>·2H<sub>2</sub>O (60 mmol) is added to a suspension of 5-chloro-8-hydroxy-7-<sup>125</sup>I]iodoquinoline derivative (60 mmol) in methanol and the mixture is stirred for 19 hours at room temperature. The product is filtered and washed with methanol followed by petroleum ether and it is left to dry in the  
25 dryer.

METHOD B: Radioiodination of the 5-chloro-8-hydroxyquinoline zinc chelator.

30 A solution of 5-chloro-8-hydroxyquinoline zinc chelator in 30 uL of ethanol is added to 10 mCi of [<sup>125</sup>I]sodium iodide (4.5 ug, 330 Ci/mmol, 30 nmol) in 0.001 N NaOH (30 uL). A solution of chloramine T (13 ug, 50 nmol) in water (10 uL) is added. The mixture is heated to 60°C for 45 minutes. 100 uL of 0.1 N NH<sub>4</sub>Cl are added and extracted with ether. The reaction  
35 product is analysed by thin layer chromatography (Silica gel,

Merck F254) in the appropriate eluent.

EXAMPLE 28:

Radioiodination of the 5-chloro-8-hydroxyquinoline manganese (II) chelator.

A solution of 5-chloro-8-hydroxyquinoline manganese (II) chelator in 30 uL of ethanol is added to 10 mCi of [<sup>125</sup>I]sodium iodide (4.5 ug, 330 Ci/mmol, 30 nmol) in 0.001 N NaOH (30 uL). A solution of chloramine T (13 ug, 50 nmol) in water (10 uL) is then added. The mixture is heated to 60°C for 45 minutes. After adding 100 uL of 0.1 N NH<sub>4</sub>Cl it is extracted with ether. The obtained product is analysed by thin layer chromatography (Silica gel, Merck F254) in the appropriate eluent.

EXAMPLE 29:

Radioiodination of the 5-chloro-8-hydroxyquinoline iron (II) chelator.

A solution of 5-chloro-8-hydroxyquinoline iron (II) chelator in 30 uL of ethanol is added to 10 mCi of [<sup>125</sup>I]sodium iodide (4.5 ug, 330 Ci/mmol, 30 nmol) in 0.001 N NaOH (30 uL). After adding a solution of chloramine T (13 ug, 50 nmol) in water (10 uL), the mixture is heated to 60°C for 45 minutes. The reaction is processed by adding 100 uL of 0.1 N NH<sub>4</sub>Cl and extracting with ether. The reaction monitoring is performed by thin layer chromatography (Silica gel, Merck F254) in the appropriate eluent.

**Examples of Activity as a Biological Marker**

EXAMPLE 30:

Quantitative autoradiography in transgenic mice and control mice.

The 5-chloro-8-hydroxy-7-[<sup>123</sup>I]iodoquinoline radioactive derivative is injected in transgenic mice (Tg2576) and control mice via the jugular vein under anaesthesia using a 30 g

5 syringe. A series of controls and transgenic mice are  
examined. After 2, 4, 6 hours of the intravenous injection,  
the brains of each mouse are removed, they are quickly cooled  
with dry ice and dissected using a Mokron HM 505E cryostat  
apparatus, placing them on glass sheets and drying them at  
room temperature. For the film autoradiography the sheets are  
placed in a MICROM optical densitometer obtaining greyscale  
images of the distribution of the radiopharmaceutical in the  
histological sections. Measured optical density is expressed  
10 as a percentage (%) of the maximum. Images were obtained with  
an optimal detection of amyloid plaques of the mice brains.

EXAMPLE 31:

MicroPET images of transgenic mice

15 All the animal experiments were performed according to  
the established animal protocol. The transgenic mice (Tg2576)  
were anaesthetised via intravenous injection with a 40 uL  
solution of ketamine and xylazine (4:1) before the injection  
of the tracer radiolabelled with  $^{18}\text{F}$ : 5- $^{18}\text{F}$ -fluoro-8-hydroxy-  
20 7-iodoquinoline. The mice were placed in supine position and  
scanned using microPET. A dynamic microPET study was performed  
using 15 planes and 15 to 16 frames (dynamic sequence: 6 x 30  
s, 4 x 1 min, 2 x 5 min, 2 x 10 min, and 1-2 x 20 min) for a  
total acquisition time of 55-77 min. Images were reconstructed  
25 using the appropriate algorithms (Filter-back projection  
algorithm) and a cut-off frequency of 0.5. Images were  
reconstructed by means of a filtered iteration resulting in an  
image resolution of 1.8 mm and a volumetric resolution of  
about 6 mm<sup>3</sup>. Transverse planes were reoriented to obtain the  
30 coronal and axial sections of the mice brains. Regional uptake  
is expressed in activity per gram of tissue. The images showed  
an adequate detection of the amyloid plaques in mice brains.

EXAMPLE 32:

35 SPECT images of transgenic mice

The study was performed using transgenic mice (Tg2576) in groups of 5 animals for each time value. The tracers (with SPECT-detectable radionuclides) (1-5 uCi) dissolved in 100 uL of PBS or in the appropriate solvent were injected via the tail vein.

SPECT images were obtained after 4.5 hours from the administration of the 5-chloro-8-hydroxy-7- $^{123}\text{I}$ iodoquinoline tracer by means of a double-head camera (MULTISPECT II, Siemens Medical Systems) equipped with parallel low energy high resolution collimators, using an energy window centred on the photopeak energy of the corresponding radionuclide (159KeV for  $^{123}\text{I}$ ).

$^{57}\text{Co}$  sources were used which were placed on the head of the animal in order to aid reconstruction by means of anatomical references. 120 projections were obtained in a 360 degree turn, acquired in a 64 x 64 matrix. Two simultaneous acquisitions are performed, one using a window of 20% of the energy centred at 159 KeV for  $^{123}\text{I}$  and another of 4% of the energy centred at 122 KeV for the  $^{57}\text{Co}$  labels. Total exploration time was about 40 minutes. The SPECT images were reconstructed using a filter-back projection algorithm. The attenuation correction was performed using the Chang algorithm.

The semiquantitative analysis of the uptake was performed comparing the average/pixel counts in the region of interest with the average/pixel counts in control regions. The results were compared with those obtained in the macroautoradiographies. The images showed the distribution of the amyloid plaques in the mice brains.